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Development of Genomic SSR for the Subtropical Hardwood Tree *Dalbergia hupeana* and Assessment of Their Transferability to Other Related Species

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Abstract: *Dalbergia hupeana* Hance (*D. hupeana*) is a precious hardwood tree of the genus *Dalbergia*. It is one of the few species widely distributed within subtropical areas and is important for timber production and forest restoration. At present, there is little published genetic information on *D. hupeana*. Therefore, we performed a genome survey using next generation sequencing (NGS) and developed a set of novel genomic SSR (gSSR) markers from the assembled data, and assessed the transferability of these markers to other *Dalbergia* species in Asia. The results of the genome survey show the genome size of *D. hupeana* to be about 664 Mb and highly heterozygous. The assembly of sequencing data produced 2,431,997 contigs, and the initial assembly of the NGS data alone resulted in contig N50 of 393 kb with a total of 720 Mb. A total of 127,742 perfect SSR markers were found in the assembled contigs. A total of 37 highly polymorphic and easily genotyped gSSR markers were developed in *D. hupeana*, while the majority of gSSR markers could be successfully transferred to nine other *Dalbergia* species in Asia. The transferability rate of gSSR markers was highest in *D. balansae*, which is more closely related to *D. hupeana*. Seven gSSR markers were able to be amplified in all tested species. In addition, a preliminary assessment of the genetic diversity of three tree species in the *Dalbergia* genus suggested a high level of genetic diversity within populations distributed in the subtropical area in China. However, the determination of the global status of their genetic variation still requires further and more comprehensive assessment. Our findings will enable further studies on the genetic diversity, phylogenetics, germplasm characterization, and taxonomy of various *Dalbergia* species.

Keywords: *Dalbergia hupeana*; genome survey; genomic SSRs; cross-species transferability; genetic diversity



Citation: Li, C.; Zheng, Y.; Liu, Y.; Lin, F.; Huang, P. Development of Genomic SSR for the Subtropical Hardwood Tree *Dalbergia hupeana* and Assessment of Their Transferability to Other Related Species. *Forests* **2021**, *12*, 804. <https://doi.org/10.3390/f12060804>

Academic Editor: Gancho Slavov

Received: 11 May 2021

Accepted: 10 June 2021

Published: 18 June 2021

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1. Introduction

The genus *Dalbergia*, a member of the Fabaceae family, contains over 250 species distributed across pan-tropical areas of the world [1]. Most species have important economic value because their hardwood is a precious timber in addition to the application of trunk root extracts in the medical field, e.g., as a source of flavonoids and terpenoids. For these reasons, the natural resources of the *Dalbergia* species have been decreasing in recent decades [2]. The genus *Dalbergia* was listed in CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), and more than one-third of *Dalbergia* species are considered endangered or critically endangered and included on the red list of the IUCN (International Union for Conservation of Nature), suggesting these species are facing different threats [3,4]. In China, 29 species of the genus *Dalbergia* were recorded in Flora of China; 14 of these species are endemic, and one has been introduced [5]. A few

studies have focused on the genetic diversity of some *Dalbergia* species [6–11]; however, many species have not been studied and the limited availability of molecular markers may be one of the primary reasons for this.

Dalbergia hupeana Hance (*D. hupeana*) is a large-sized tree species widely distributed in the subtropical areas of China and South Korea [12,13]. This species is a precious hardwood and a broad-leaved tree of economic and ecological value, with applications in timber production and landscape restoration. However, the natural resources of this species are decreasing, and its habitat is also becoming increasingly fragmented, according to a previous report [14]. Genomic knowledge on *D. hupeana* and an assessment of its genetic resources are lacking.

Molecular markers are a powerful tool for characterizing interspecific and intraspecific genetic variation [15–19]. Thus, cases where the genetic diversity of trees has not been assessed primarily result from a lack of effective molecular markers [20]. Therefore, the development of molecular markers is a key component of the fundamental work required for forest genetic resources (FGR) conservation and management. Simple sequence repeat (SSR) markers are powerful, effective, and economical for the assessment of co-dominant inheritance, with good reproducibility [21,22]. The development of next generation sequencing (NGS) techniques has resulted in a revolution in the field of de novo whole genome assembly and multi-omics of plants [23], and developing genomic or genic SSRs from NGS data is both feasible and affordable [24–26].

In the described study, we aimed to uncover genomic knowledge and develop effective SSR markers for *D. hupeana* by utilizing de novo sequencing to survey and characterize the *D. hupeana* genome. We then screened and verified a set of genomic SSR (gSSR) by PCR and capillary electrophoresis. Additionally, in order to potentially broaden the scope of application of these markers, their transferability and degree of polymorphism were examined in other related species in the *Dalbergia* genus and representative populations.

2. Materials and Methods

2.1. Plant Materials

A diploid seedling of *D. hupeana*, which was collected in Anhui province, was used to perform de novo sequencing. The information of other plant materials in the *Dalbergia* genus is shown in Table 1. Their fresh leaves were collected, dried, and stored in silica gel at 4 °C. The voucher specimens of *Dalbergia* species were preserved in the Research Institute of Forestry, Chinese Academy of Forestry. Photographs of the studied species of *D. hupeana* and its leaves and fruits are shown in Figure 1. Total genomic DNA was extracted from leaf tissue using a DNA extraction kit (NuClean Plant Genomic DNA Kit, CWBIO) according to the manufacturer's protocol. The concentration of total genomic DNA was examined using a microplate spectrophotometer (Molecular Device, Sunnyvale, CA, USA). The quality of total genomic DNA was verified by electrophoresis on 0.8% agarose gels.

Table 1. Information of plant materials of the *Dalbergia* genus collected in this study.

Name	Biotype	Location	No. Individuals
<i>D. balansae</i>	tree	Yingjiang, Yunnan, China	29
<i>D. cultrata</i>	tree	Puer, Yunnan, China	2
<i>D. hupeana</i>	tree	Jiangyou, Sichuan, China (JY)	30
<i>D. hupeana</i>	tree	Xixiang, Shanxi, China (XX)	30
<i>D. hupeana</i>	tree	Dujiangyan, Sichuan, China (DJY)	30
<i>D. mimosoides</i>	vine	Tongren, Guizhou, China	2
<i>D. odorifera</i>	tree	Jiulongling, Fujian, China	2
<i>D. polyadelpha</i>	tree	Longling, Yunnan, China	29
<i>D. sissoo</i>	tree	Yunnan, China	2
<i>D. yunnanensis</i>	shrub	Panzhihua, Sichuan, China	2
<i>D. cochinchinensis</i>	tree	Jiulongling, Fujian, China	2
<i>D. oliveri</i>	tree	Jianfengling, Hainan, China	2

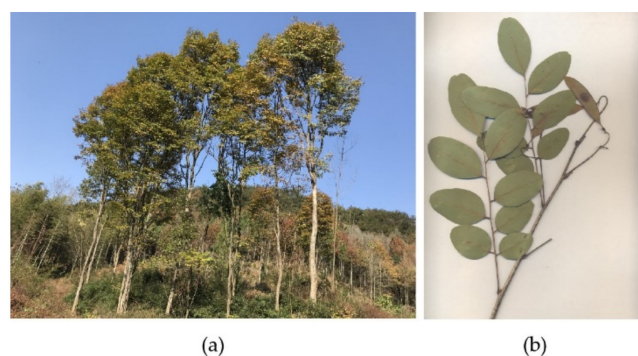


Figure 1. Photographs of the studied species *D. hupeana* depicting its (a) habitat in China and (b) leaves and fruits.

2.2. De Novo Genome Sequencing

Three genomic paired-end libraries with 350 bp insertions were constructed using the Illumina HiSeq platform (Illumina, San Diego, CA, USA) and a diploid seedling of *D. hupeana*, following the guidance of the standard procedure at Biomarker Technologies Co., Ltd. (Beijing, China). Clean reads were obtained after the filtering and correction of the sequence data, and were relatively accurate for estimating the genome size, heterozygosity rate, and percentage of repetitive content.

2.3. Genome Survey and Assembly

Genome size was estimated using the total length of sequence reads divided by the sequencing depth. The sequencing depth of *D. hupeana* was calculated based on the k-mer ($k = 21$) analysis using a Lander–Waterman algorithm, and we estimated the genome size, heterozygosity rate, and percentage of repetitive content using the k-mer method [27]. The genome assembly based on the NGS refined reads ($k\text{-mer} = 55$) was performed using SOAPdenovo with default parameters [28].

2.4. Identification and Verification of gSSR Markers

The sequence-repeat search mode in MISA software (Saxony-Anhalt, Germany. <http://pgrc.ipk-gatersleben.de/misa/misa.html>, accessed on 12 February 2021) was used to mine candidate SSR markers from all contigs of the draft *D. hupeana* genome. The following search parameters were set for discovering SSR markers: di-, tri-, tetra-, penta-, and hexa-nucleotide motifs with a minimum of 6, 5, 5, 5, and 5 repeats, respectively. Then, primer pairs that flanked the SSRs were designed using Premier v5.0 (Palo Alto, CA, USA) with the parameters of a 100–300 bp final product length, 18–22 bp primer size, 40–60% GC content, and 55–62 °C annealing temperature, with an optimum annealing temperature of 60 °C. A total of 200 randomly selected candidate gSSR loci were used for preliminary screening, and the corresponding primers were synthesized by Majorbio Biotech (Beijing, China). A total of 8 individuals of *D. hupeana* from different locations were selected to test the gSSR markers for polymorphism. PCR amplifications were performed in 20 µL reaction volumes as follows: 10 µL of 2 × Taq MasterMix (Aid Lab, Beijing, China), approximately 50 ng of DNA, 5 pmol of reverse primer and 5 pmol of forward primer with the 5' end labeled with fluorescent dyes (FAM, HEX, ROX, or TAMRA; Ruibiotech, Beijing, China), and sterile double-distilled water added to 20 µL. PCR reactions were performed with a touchdown profile with an initial denaturation at 94 °C for 5 min; 10 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 30 s, with a 1 °C decrease in the annealing temperature of each cycle; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 10 min. The PCR products were detected by an ABI 3730XL capillary electrophoresis analyzer with a GeneScan-500LIZ size standard. Fragments were genotyped for their presence/absence at each locus, and the allele sizes were scored using GeneMaker v2.2.0 (SoftGenetics LIC, State College, PA, USA) and manually checked twice to reduce genotyping errors.

2.5. Transferability to Other *Dalbergia* Species

Cross-species amplification was tested in another nine *Dalbergia* species (Table 1) using the polymorphic gSSR markers. The total genomic DNA was extracted from two individuals for each species. PCR amplification was performed, and the methods were conducted as described under the section “Identification and Verification of gSSR markers”.

2.6. Assessment of Genetic Diversity in Different Species

Five populations in the *Dalbergia* genus (three representing *D. hupeana*, and one each for *D. balansae* and *D. polyadelpa*) were used to test the effectiveness of the developed gSSR markers in the assessment of genetic diversity. The following genetic diversity parameters were calculated using GenALEX v6.502 (Canberra, Australia) [29,30]: the number of observed alleles (N_a), the number of effective alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), Shannon’s information index (I), and unbiased expected heterozygosity (uH_e). The polymorphism information content (PIC) of gSSR markers was calculated using CERVUS v3.0.4 (London, UK) [31].

3. Results

3.1. Genome Survey

Three libraries of paired-end sequences with 350 bp short inserts of individual of *D. hupeana* were constructed. A total of 120.24 Gb of clean data were obtained, which correspond to an approximately 180-fold coverage of the estimated genome size. The Q20 and Q30 were 97.11% and 92.16%, respectively. All clean data were used for assembly and k-mer analysis; the results show the number of k-mers was 100,590,170,915 and the peak of the depth distribution was 151 for the 21-mer frequency distribution (Figure 2a). The genome size of *D. hupeana* was estimated as 664 Mbp, the heterozygosity rate was calculated to be 1.00%, the repeat rate was estimated as 42.84%, and an average of 34.93% GC content was determined (Figure 2b). Based on the k-mer curve distribution, a k-mer size = 55 was chosen for assembly with the default parameters in SOAPdenovo, and a total of 2,431,997 raw contigs were produced to assemble a final draft genome of 720 Mb with contig N50 size of 393 kb.

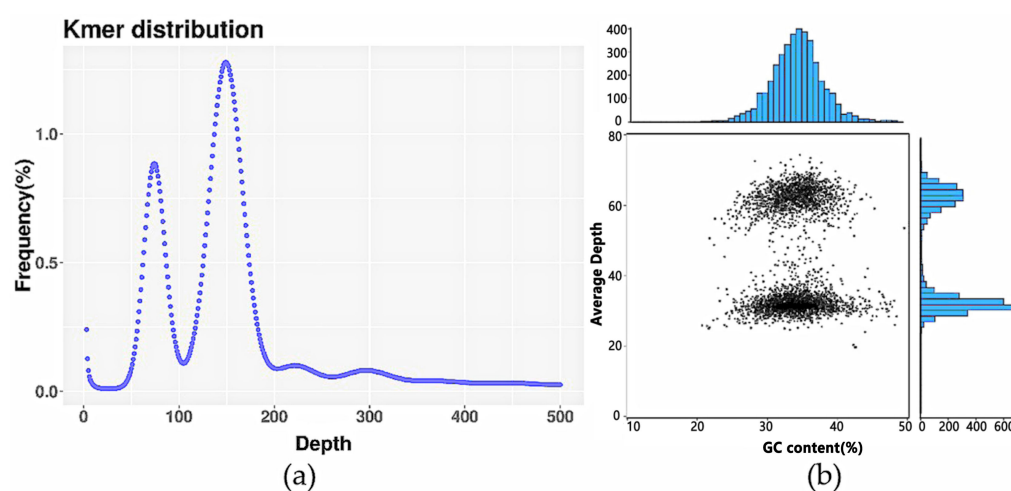


Figure 2. Diagram showing k-mer distribution, GC content, and average sequencing depth: (a) 21 k-mers for estimating the genome size of *D. hupeana*. (b) For scatter plots, the X-axis represents GC content and the Y-axis represents the average depth. For the histograms, the X-axis represents the GC content and the Y-axis represents the frequency distribution of sequencing depth.

3.2. Development and Verification of gSSR Markers

After filtering, a total of 127,742 perfect SSR markers were found in the assembled contigs. The most abundant motifs were di-nucleotide repeats (88,999), accounting for 69.67%, followed by tri-nucleotide repeats (34,536), accounting for 27.03%. Finally, tetra-nucleotide and penta-nucleotide repeats accounted for 2.41% and 0.61%, respectively. Then, 200 pairs of primers were randomly selected to test the success rate of amplification, and eight individuals of *D. hupeana* from different sites were randomly chosen for the detection of polymorphisms. As a result, most of the primers yielded clear amplification, with nearly a 57% success rate. However, 37 pairs of primers were successful in revealing abundant polymorphisms, showing clear allelic bands; the characteristics of these gSSRs are shown in Table 2. These gSSR markers were further utilized for population genetic analysis of three wild populations of *D. hupeana*, and their transferability to other *Dalbergia* species was tested.

Table 2. Characteristics of 37 gSSR markers designed for *D. hupeana* in this study.

Name	Repeat Motif	Forward Primer Sequence (5'–3')	Reverse Primer Sequence (5'–3')	T _m (°C)	Size Range (bp)	Fluorescence Dye
Dhup14	(TCT)5	TCACAAAGGTATGCATTTC	TATACGCTTGGCATTTCAG	59	207–219	FAM
Dhup61	(GGA)11	GGTCGAAGTGGGAATCGAAG	ATTCTCCCTCCCTTGCTCAT	60	102–120	FAM
Dhup64	(TTA)9	AGTTAGGTGCACACACAGCT	TCTATGCCGATGTTTCCCTC	59	158–188	FAM
Dhup70	(TTA)14	AAGCAACGGAACAATATGAA	TTATATCACCACCGCACA	59	203–248	FAM
Dhup78	(ATT)10	AGTATCCATCCCTGGGTTCC	GTGGGTGGCGTACTGTTTT	59	257–284	FAM
Dhup89	(TA)20	TACCGTTGGATGTGATGGTG	ATGCTTCTGCACCCAGAA	59	143–177	FAM
Dhup90	(AAT)12	TCCGATATGCAAAACATGAGC	AATCACGTGGCTTTGATTCC	59	222–255	FAM
Dhup104	(AG)14	TGGACCAACCCATGATAGTGA	GGAACAATAGAAACAAACACGG	59	108–154	FAM
Dhup106	(AT)14	CATTGGCATATAACATGTCGG	GCAACAGTGTAACTGATGTGCT	59	115–165	FAM
Dhup108	(TC)14	CCCCCTTAAACTTGCTTCA	CTGATGAGTGGGAAAACGGT	59	117–153	FAM
Dhup114	(GGC)8	TATTTTGGGATCGTTGCTG	GCTCGCGTCACTGAGACTT	59	116–143	FAM
Dhup115	(AAG)10	TGAAAATGGAGAATGGGGAG	GATACACACGGGAAAATCGG	59	115–142	FAM
Dhup116	(AAT)8	ATTTCATTCTCTCGGCTGT	CAAATTTGTGAGCACATGATGA	59	127–157	FAM
Dhup118	(AAG)7	TTGATCCAAGGGCGAGTAAC	GTGGGCTCTGTGTGTGTA	59	128–149	FAM
Dhup120	(AAT)8	TGAATTTTCTCGCAAGAGC	TTCTTCTTCTCACCAGAC	60	124–154	FAM
Dhup122	(AAT)8	GAATGAGCTGATGGTGAAGGA	ACATTAATACCTTATCTCAATTCTG	58	133–154	FAM
Dhup123	(AAG)7	TGAGTGAGAGAAAGATAGACG	CCAACGAAACCTGTTTACA	59	135–168	FAM
Dhup125	(AAG)7	TGCTCGTTTCTCTCTCTATCC	TTGTGCGCTACCAACAGAG	60	133–157	FAM
Dhup129	(TA)14	TGTGGTGCATGAAGACTTGG	GCCAGCTATGTGTGAAGTGC	59	174–188	HEX
Dhup130	(GA)13	AAGCAGCAATTAATTTCTTGA	AGAGAGTGTGTGGTGTGGG	59	228–276	HEX
Dhup132	(AT)14	TTGCGTGCATTTGATTTGT	ACCATCTGGGCACTGATAGC	59	195–233	HEX
Dhup133	(AT)14	CAATACATATGCAACAAATTCAAA	CCCGAATGAGAAATAGGAAA	58	199–243	HEX
Dhup139	(AAT)8	CCCCGGCACTCTCTATATCA	ACTGGTATCTTGTGCTCT	60	181–199	HEX
Dhup141	(TTG)8	CCACCGTGAAAGTAGACCAAG	CTTCCCTTGAGGACCTAC	59	187–205	HEX
Dhup142	(CAA)10	TTTGAGGCCAACCTTCAAAT	ACGAGTGGAGTGAAAGTCAA	59	200–221	HEX
Dhup144	(AAT)8	AACAAATTCAAACCAACCGA	TCCAAAGTAGGCTTGAAAAA	59	184–217	HEX
Dhup153	(AG)14	ACTTGTGTACAGTTCTGCCA	CTGCTTCTCTGCTTCTGCT	60	251–293	ROX
Dhup159	(TA)14	TTTCAGATGCTAGAGTGTGTG	CGTGTGCCTAAATTTCTAACCG	58	206–250	ROX
Dhup163	(TTG)8	ACGAAACCTTAGCCCTTGT	CAACGAACTCTTGAAAGGC	59	228–255	ROX
Dhup164	(AAG)7	AATTGCACAAACATCCAGCA	CGGATTAATCTCCGAAAACGA	60	233–254	ROX
Dhup167	(AAT)8	AAAGGCTGAGAGGAAAAGGC	TCTAAATTAAGGATTTTCAGTGTAT	57	232–250	ROX
Dhup171	(GAG)8	GTGCGGTACCTTCATCAT	GTCACGATCAAAAGTCGGTT	59	228–249	ROX
Dhup181	(AT)14	CCACCAGCAAAATAGCTCACA	GGATTCCGAACCTTGGAAGAA	59	271–293	TAMRA
Dhup183	(AT)14	CCAGACATGGTGGTTTCAGA	CGATATTACATTTTAAAGACTTGG	58	272–324	TAMRA
Dhup185	(AT)14	GTGCCAAGTACAACCTGCGAA	TCACACTTACCCACCTTATGA	59	258–308	TAMRA
Dhup191	(AAT)8	GTGATGGTCACGTCATTTC	TTTCTACCCGCTTAACTT	59	279–303	TAMRA
Dhup194	(AAT)8	AACGATTGATCTTGGTCATGT	TGTCGGTGTCTATCAATTCCC	59	247–292	TAMRA

Note: T_m, annealing temperature.

3.3. Transferability of Developed gSSR Markers to Other *Dalbergia* Species

A total of 37 polymorphic gSSR markers were developed and verified in *D. hupeana*. All of these gSSR markers were then tested for amplification in nine *Dalbergia* species for the assessment of cross-species transferability. Among the 37 tested primer pairs, seven gSSR markers (Dhup14, Dhup61, Dhup114, Dhup120, Dhup139, Dhup141, Dhup164) were able to be amplified in all nine tested species, and two gSSR markers failed to yield positive amplicon in any of the other tested *Dalbergia* species (Table S1). The rates of successful amplification in other *Dalbergia* species were recorded as between 40.54% and 70.27%. A higher number of gSSR marker sets were successfully amplified in *D. balansae* (26), *D. oliveri* (23), *D. polyadelphae* (21), and *D. cochinchinensis* (20), with the lowest in *D. yunnanensis* (15) (Figure 3, Table S1).

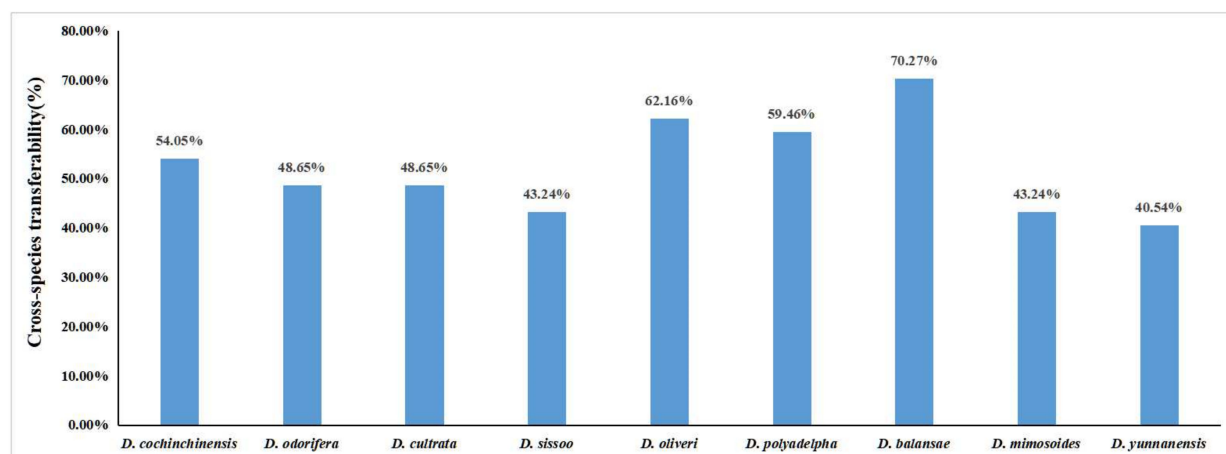


Figure 3. Cross-species transferability of gSSR markers developed in *D. hupeana*.

3.4. Diversity of gSSR Markers in *Dalbergia* Populations

In *D. hupeana*, the number of alleles (N_a) ranged from 2 (Dhup129, DJX) to 18 (Dhup106, XX); the effective number of alleles (N_e) varied from 1.342 (Dhup129, DJX) to 10.651 (Dhup133, JY). The Shannon's information index (I) varied from 0.423 (Dhup129, DJY) to 2.573 (Dhup133, JY). The observed heterozygosity (H_o) varied from 0.033 (Dhup142, DJY) to 0.967 (Dhup78, JY); the expected heterozygosity (H_e) varied from 0.255 (Dhup129, DJY) to 0.906 (Dhup133, JY). The value of the polymorphism information content (PIC) varied from the minimum of 0.222 (Dhup129, DJY) to the maximum of 0.899 (Dhup133, JY). The average value of PIC among the three populations varied from 0.618 (JY) to 0.711 (XX), and the proportions of gSSR with moderate ($0.25 < \text{PIC} < 0.5$) or high polymorphism ($\text{PIC} > 0.5$) was 81.08% (DJY), 78.38% (JY), and 86.49% (XX), respectively. These findings indicate that the majority of developed gSSR markers showed high polymorphism in *D. hupeana* populations (Table S2).

A total of 26 of the newly developed gSSR markers were effectively amplified, and all of these gSSR markers were polymorphic in the *D. balansae* population ($n = 27$). The number of alleles (N_a) varied from 3 (Dhup164) to 15 (Dhup108 and Dhup104), with an average of 7.038. The mean value of PIC was 0.604, ranging from 0.278 (Dhup14) to 0.904 (Dhup108); 18 gSSR markers were shown to be highly informative (Table S3). The observed heterozygosity (H_o) ranged from 0.074 to 0.885, and the expected heterozygosity (H_e) ranged from 0.294 to 0.911, with a mean of 0.467 and 0.635, respectively (Table S3).

A total of 22 of the newly developed gSSR markers proved to be effectively amplified in the *D. polyadelpha* population ($n = 27$). A total of 21 gSSR markers were polymorphic, except Dhup120. The number of alleles (N_a) varied from 2 (Dhup78, Dhup144, Dhup181 and Dhup183) to 14 (Dhup122) with an average of 5.091 per locus. The mean value PIC was 0.471, ranging from 0.071 (Dhup70) to 0.879 (Dhup122). A total of 11 gSSR markers were shown to be highly informative in *D. polyadelpha*. The observed heterozygosity (H_o) ranged from 0.037 to 0.889, and the expected heterozygosity varied from 0.072 to 0.889, with a mean of 0.399 and 0.512, respectively (Table S3).

4. Discussion

The *Dalbergia* species are good sources of precious timber; a few tree species have been identified as rosewood, and genetic research has been performed on them. The genetic diversity of endangered species and their phylogenetic relationships have been unveiled through studies using molecular markers and DNA sequences [6–11]. However, many species in the *Dalbergia* genus have not been investigated, especially subtropical species, which are good sources of hardwood and also face threats from climate change and human activity [32–35]. The development of effective and powerful molecular markers

will be conducive to facilitating research on these species. SSR markers are one of the most powerful DNA markers for genetic research because of the co-dominant inheritance of SSRs and their amenability to genotyping. The development of NGS techniques has made it easier to acquire more genetic information and develop SSR markers. In this current study, the genomic information of *D. hupeana* was obtained using NGS. The results indicate that the *D. hupeana* genome has high heterozygosity with a medium number of repetitions of medium size. Combined with Hi-C technology, the most up-to-date third-generation sequencing (Circular Consensus Sequencing, CCS) has the potential to assemble a high-quality and chromosome-level reference genome [36,37]. Using the acquired sequence information, genomic SSRs were mined, characterized, and evaluated in *D. hupeana*. Dinucleotide repeats were observed to be the most abundant type of repeat motif in the genome. Trinucleotide repeats are also one of the most abundant repetition motifs in genes for tree or fruit species because they are less likely to lead to frameshifts in the coding sequence [38]. The PIC value is a considerable parameter showing the power of a molecular marker. It is generally acknowledged that molecular markers with $PIC > 0.5$ are defined as highly polymorphic [39]. Most of the developed gSSR markers show high polymorphism in *D. hupeana* according to the PIC value of each locus. This indicates that these gSSR markers have good potential to detect genetic variation, being useful for further population genetic analyses of this species.

Previous reports indicate that SSR markers are transferable among closely related species in plants [40,41]. In the *Dalbergia* genus, several SSR markers from *D. nigra* and *D. monticola* were transferrable to other *Dalbergia* species in South America [42]. It is generally known that the amplification conditions affect the efficacy and fidelity of PCR [43]. In this study, the majority of high polymorphic gSSR markers could be successfully transferred from *D. hupeana* to other *Dalbergia* species in Asia. Only two gSSR markers in *D. hupeana* could not be amplified in the other nine species, and seven gSSR markers could be amplified in all tested species. The transferability rates ranged from 40% (*D. yunnanensis*) to 70% (*D. balansae*). The transfer success rate seems to be related to consanguinity in accordance with the inferred phylogenetic relationships in the *Dalbergia* genus [34,44–46]. These results indicate high conservation of the primer binding sites in the genome of the 10 selected *Dalbergia* taxa assayed in the present study, and these gSSR markers may have great potential in phylogenetic and evolutionary studies of *Dalbergia* populations. Finally, we selected natural populations from three *Dalbergia* species to assess their genetic diversity using the developed gSSRs. The results show that, compared to *D. hupeana*, the average number of alleles (N_a) in *D. balansae* was similar, while that of *D. polyadelpha* was lower. A higher expected heterozygosity (H_e) may indicate abundant genetic diversity within populations of *D. hupeana* and *D. balansae*; these values were similar to those of *D. oliveri* (mean $H_e = 0.73$) [47]. However, H_o was lower than H_e , suggesting these populations may have experienced isolation, genetic drift, or inbreeding, though further population genetic studies would be needed before making any conclusions.

5. Conclusions

We performed a genome survey in *D. hupeana* to gain knowledge on the structure of its genome. The k-mer analysis suggested that the genome of *D. hupeana* is medium sized and highly heterozygous. The assembled data were also utilized to screen and develop a set of novel gSSR markers, which showed high polymorphism and ease of genotyping. The majority of gSSR markers could be successfully transferred to other *Dalbergia* species and their populations in Asia. These findings will enable further studies on genetic diversity, phylogenetics, germplasm characterization, and taxonomy of various species of the genus *Dalbergia*.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/f12060804/s1>, Table S1. Cross-species transferability of developed gSSR markers in different *Dalbergia* species, Table S2. Parameters of genetic diversity for *D. hupeana* populations at loci level, Table S3. Parameters of genetic diversity for *D. balansae* and *D. polyadelpa* populations at loci level. Another Supplementary Materials includes all the electropherograms and DNA matrix in this study.

Author Contributions: Conceptualization, Y.Z. and P.H.; methodology, C.L., Y.L. and P.H.; formal analysis, C.L.; investigation, C.L. and Y.L.; resources, F.L.; writing—original draft preparation, C.L. and P.H.; writing—review and editing, Y.Z. and P.H.; supervision, Y.Z.; funding acquisition, Y.Z. and P.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (No.31761143002; No.3207141079) and Special Funds for the Laboratory of Forest Silviculture and Tree Cultivation, National Forestry and Grassland Administration (No. ZDRIF201713).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: We thank the National Forestry and Grassland Genetic Resources Center (Beijing, China) for support related to the plant material.

Conflicts of Interest: The authors declare no conflict of interest.

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